Foreign Animal Disease Report

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Emergency Field Investigations

Foreign Animal Disease Investigations. During the first quarter of Fiscal Year (FY) 1989, veterinarians from the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, conducted 39 investigations of suspected foreign animal diseases to eliminate the possibility that exotic diseases were introduced into the United States. All investigations were negative for exotic diseases. Foreign Animal Disease Diagnosticians (FADD's) are required to officially report all investigations of suspected foreign diseases.

Avian Influenza (AI). Avian Influenza, highly pathogenic H_5N_2 AI, was last confirmed in the United States on July 1, 1984, in Virginia. Since then, several flocks have contained serologically positive birds and several isolations of AI viruses have been made. Isolates of H_5N_2virus obtained after 1984 during surveillance activities were not pathogenic for chickens in the laboratory. A nationwide survey was conducted during 1986 and 1987, to determine if carriers of the virus were moving through live bird markets. Results of the survey confirmed the absence of highly virulent AI virus pathogenic for chickens.

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Live bird markets were sampled in New York in January 1988, and those in New Jersey during February 1988. The results of the survey were negative for H_5N_2 AI virus. A total of 114 New Jersey bird owners were surveyed on 35 premises. All results were negative. Other States in the Northern Region of VS will be surveyed during March and April 1989.

A flock in Florida was found serologically positive for Type H_5N_2 AI during December 1988 after sentinel birds seroconverted. Attempts to isolate the virus were unsuccessful. Although clinically normal, the flock was promptly depopulated. The premises were cleaned and disinfected and sentinel birds were replaced.

During 1988, in Minnesota, 243 turkey flocks and 188 premises were serologicaly positive for one of the following AI serotypes: H_7N_9, H_2N_2, H_9N_2, H_2N_2, H_9N_2, H_5N_6, H_7N_6, and H_8N_4. In two instances, the infection spread to chickens and, in another, to pheasants. Except for these three occurrences, the antibodies were in turkeys. The last time influenza spread from turkeys to chickens was reported in 1978. (Dr. John L. Williams, (301) 436-8073)

Emergency Programs Activities

Training. Foreign animal diseases (FAD) training for State, Federal, military, and university veterinarians will be conducted by the Emergency Programs Staff during September 1989. Training for professors of infectious diseases and laboratory personnel will follow at

the Plum Island Animal Disease Center in November. The training of professors provides for the dissemination of FAD information to future veterinarians as they teach in colleges of veterinary medicine.

Two seminars for FADD's are being planned this year to provide knowledge of current investigative and diagnostic techniques to previously trained FADD's. A Wildlife Diseases Seminar is also scheduled for August-September 1989.

Recorded Emergency Animal Disease Information (READI) System. Field tests of the READI System were conducted in FY 1988, using a modified Regional Emergency Animal Disease Eradication Organization (READEO). The testing indicated that the READI System is fully operational but inadequate in speed and portability to meet the increasing needs of a disease emergency. The major problem encountered was the slowness of the system. This was due both to the READI software and the hardware used. After much effort to correct this problem, it was decided that software replacement was the best answer to speed up the system. READI was subsequently reprogrammed in APHIS' standard ORACLE; the new program requires the use of larger amounts of computer memory and modern microprocessors. Consequently, the new program will not run on the old READI personal computers.

The probability of placing the system in the field is improving and will provide quick response time to input data. Attempts are being made to procure up-to-date hardware equipment to run the updated software.

Data from field investigations of suspected emergency animal diseases continues to be entered and maintained in the online READI system.

Biosecurity Awareness. Close liaison has been maintained with the Mid-Atlantic Cooperative Extension Service and other agencies and organizations for the past several months to develop brochures and program aids on disease prevention and biosecurity. The eight video presentations on biosecurity that are being developed for use in the poultry industry are titled: "What is Biosecurity?;" "Broiler Operations;" "Live Bird Markets;" "Egg Laying;" "Hatcheries;" "Feed Mills and Transportation;" "Turkey Operations;" and "Game Birds." An exhibit on disease prevention in the poultry industry has also been developed and is being used at local, State, and national poultry meetings to further increase public and industry awareness.

Smuggled Birds. Spring is the time of year when an increase in the smuggling of birds can be expected in the United States. Most of the estimated annual influx of 25,000 illegal birds enter from the beginning of January through mid-May, coinciding with the normal hatching season for wild birds. Although the Mexican border is the most likely location for bird smuggling, many of these birds reportedly originated beyond Mexico, particularly from Central and South America. (Dr. John L. Williams, (301) 436-8092)

African Horse Sickness in Spain

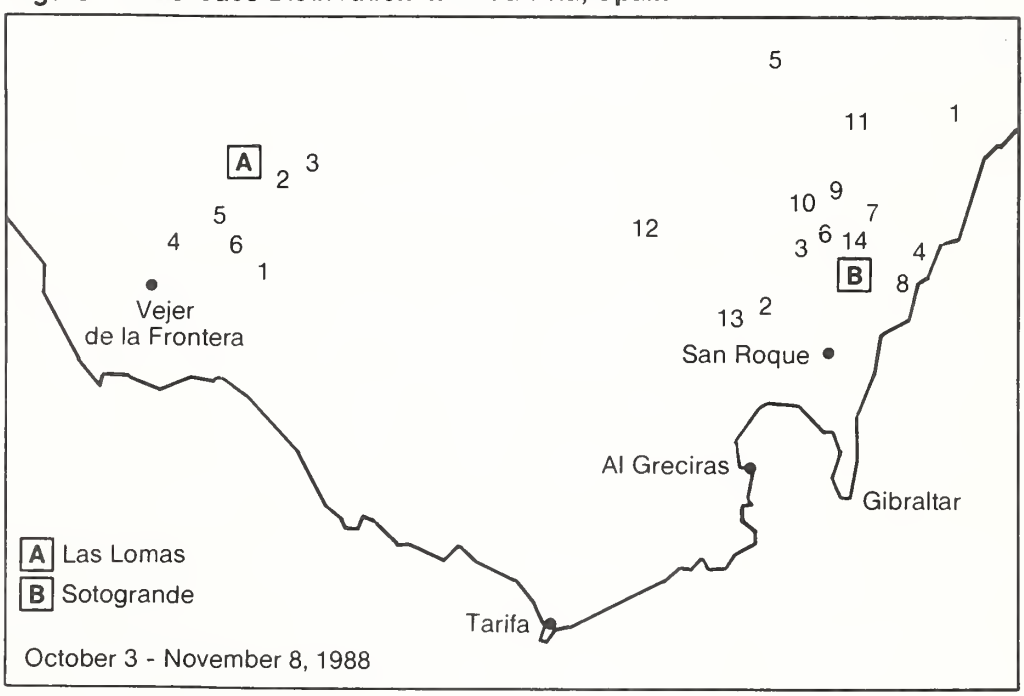
Case History. On October 3, 1988, a polo horse died on a farm in Las Lomas in the Province of Cadiz in Andalucia. Three more polo horses were sick when the veterinarian arrived. On October 5, one horse died at the Polo Club Sotogrande in Eastern Cadiz. Horses from Las Lomas had been transported to competitions at Sotogrande on several weekends immediately preceding the outbreak. Within 8 days, 20 horses had died at those 2 foci on adjacent farms. Equine deaths on adjacent farms were attributed either to movement of animals to and from Las Lomas and Sotogrande or to residing within the flying range of infected *Culicoides* vectors (see adjacent map/table).

Table 1. Preliminary AHS Case Information in Andalucia, Spain

Farm*		Date of First Death**	No. Equines on Farm	No. Dead Horses	No. Horses Sacrificed
A.	**Las Lomas** (pastures)	10/03	51	15	7
0.	Las Lomas (stables)				
1.	Jandilla	10/13	6	1	1
2.	Cantaranas	10/17	2	0	2
3.	Malcocinado	10/20	1	1	0
4.	Horcajo	10/21	2	0	1
5.	Najaras	10/25	3	1	1
6.	Espartine	11/03	1	1	0
B.	**Sotogrande**	10/05	90	16	10
1.	El Cortesin	10/08	16	3	0
2.	Los Acebuches	10/08	9	2	0
3.	Las Presillas	10/12	1	1	0
4.	Torre Guadiaro	10/12	15	1	0
5.	Esparagal	10/19	38	1	0
6.	Canelo	10/19	2	0	1
7.	El Torrito	10/21	5	1	0
8.	Los Pinos	10/21	28	2	4
9.	San Enrique	10/21	23	0	1
10.	Montenegral Alto	10/21	40	1	2
11.	Manantial	10/21	4	0	2
12.	Navamatrera	10/23	2	0	1
13.	Mojones Blancos	10/24	12	1	1
14.	Los Claveles	10/25	10	0	2
TOTAL (October 3 - November 8, 1988):			361	48	36

*Numbers correspond to map (Figure 1).
**Death due to AHS or due to sacrifice of diseased horse(s).

Figure 1. AHS Case Distribution in Andalucia, Spain



The number of cases immediately adjacent to Las Lomas seem to have been exacerbated by the enclosure of the 25 polo horses at Las Lomas. Upon movement of the horses from the vector-infected pasture to the insecticide- treated enclosures, the *Culicoides* seem to have gone in search of a blood meal, thereby infecting six neighboring farms. At Las Lomas, only polo horses were apparently affected, even though work horses were present.

Occurrences of AHS in horses near Sotogrande seem attributable to their participation in the Sotogrande polo competitions. Due to the nature of polo, it is the horse that rests and not the rider. Each participating rider must take approximately 10 horses to competition: five for Saturday and five for Sunday. There are eight players per team; therefore, equine movements were rather extensive around the Sotogrande area. Fourteen nearby premises had AHS in at least one horse. Quick detection and the prohibition of equine movement probably prevented a more serious outbreak.

Control. Spanish authorities reacted quickly and efficiently to control AHS. At Las Lomas on Monday, October 3, a polo horse was found dead in its stall, with froth exuding from the nares. An alert veterinarian submitted a spleen sample and 12 heparinized blood samples from other febrile horses on the premises to the central laboratory at Algete, Madrid. The samples were inoculated into cell cultures and suckling mice (intracerebral) on October 6. Nervous signs in the mice and cytopathic effects (CPE) in cell culture were present on October 13. Serotyping by virus neutralization (VN) was achieved by October 16. The diagnosis of AHS was officially announced Monday, October 17, 1988.

Prior to laboratory confirmation and based on clinopathologic diagnosis, Spanish animal health authorities canceled all equine events within Andalucia, including the Annual Pure Breed Spanish Horse Show, which was to have been attended by Queen Elizabeth II. It also closed the Andalucian boundaries to equine traffic, ordered the slaughter of any suspicious clinical cases on affected farms, instituted daily spraying of affected premises with malathion, and requested vaccine from South Africa. After laboratory confirmation, additional control measures were undertaken, including the prohibition of movement of any horses within Andalucia and demarcation of potential vector zones. A region of southern Andalucia was designated as most susceptible to the propagation of infected vectors, with boundaries consisting of the Mediterranean to the south and topographic elevations greater than 700 m to the north. All horses within this zone were very carefully monitored either by private veterinarians or GEPE (Grupo Executor de Programas de Emergencia) veterinarians. Approximately 28,000 doses of AHS vaccine had been administered up to the time of this report.

GEPE Organization. Patterned after the Regional Emergency Animal Disease Eradication Organization (READEO) of the U.S. Department of Agriculture, the GEPE organization consisted of a coordinator and two teams of field veterinarians with each team of four veterinarians stationed at one of the two outbreak foci. These veterinarians examined animals on affected premises daily, supervised the daily monitoring of rectal temperatures of horses throughout Andalucia, ensured daily spraying of affected areas with malathion, and treatment of low-lying marshy zones with a larvacide (tenafos) every 48 hours. Also, any horses with clinical signs of AHS were sacrificed and necropsied. In addition, these veterinarians administered or supervised the administration of the Onderstepoort-modified live virus vaccine. As in 1987, the vaccine was administered in only one dose to avoid prolonged seropositive titers. The first 13,500 vaccines were polyvalent, but only the vial containing serotype IV along with three other serotypes was used. By November 4, 25,000 doses of monovalent (serotype IV) vaccine had arrived from Onderstepoort.

Vector Monitoring. A biologist was employed to trap and classify insects at any farm where AHS occurred and to assess the effects of insecticides. Insecticides were applied only to farms where *Culicoides imicola* was found by the trapping method. Samples of *C. imicola* were preserved in liquid nitrogen for later analysis for AHS virus.

Mortality Monitoring. In addition to cases from Andalucia, all suspicious horse deaths throughout the country were investigated by culturing spleen samples at the central laboratory in Algete, Madrid. This was the only laboratory in Spain which was equipped to perform both suckling mouse intracerebral inoculations and cell culture inoculations for the diagnosis of AHS. The veterinarians performing this work were very experienced as a result of their participation in the 1987 outbreak. The death toll due to AHS in Andalucia, including infected animals that were sacrificed, was estimated at 160 to 165 equines.

Source of Outbreak. Several possibilities have been advanced for the origin of the 1988 AHS outbreak.

Overwintering of the virus from 1987: This possibility seems unlikely because a completely different area of the country was involved. Also, the disease did not appear at the onset of the vector season but several months later, and the 1987 outbreak appeared to have been completely eradicated, as proven by the use of sentinel animals and serologic surveillance.

Windborne spread from North Africa: Exceptionally strong winds were encountered during September 1988, blowing sand from the Sahara and locusts from the Sudan onto the southern coast of Spain. However, AHS virus serotype 4 is not known to occur north of tropical Africa, and no AHS cases have been reported in countries that are closer to tropical Africa. Consequently, this explanation seems unlikely.

Entry of Culicoides by vehicle: Each year, approximately one million migrant workers from Africa or equatorial Africa enter Europe by the straits of Gibraltar, usually during September. Most of them cross by automobile and ferry. An infected Culicoides could conceivably be carried along within the vehicle. These migrant workers move into central Europe either traveling up the coast and thereby passing close to Sotogrande, or by an inland route, near Las Lomas. Another possibility is entry through the considerable marine traffic along the Mediterranean, where infected Culicoides could be brought by boat from Africa into southern Spain.

Illegal importation of infected animal: It is possible that an infected horse may have been smuggled into Spain by private boat or plane from an endemic area in Africa.

Observations. The authors personally observed the AHS outbreak in Spain, November 1-8, 1988. They were very impressed with the rapidity and efficiency with which the Spanish authorities reacted to control the outbreak. Rather than waiting for laboratory confirmation, those officials took drastic measures to contain the disease after making a tentative clinicopathologic diagnosis. That initial effort probably served to avert a more widespread disaster. Laboratory confirmation of the diagnosis was also very rapid, with positive identification and serotyping completed within 10 days following receipt of the sample. The veterinarians involved in the diagnosis of the first few cases deserve praise. Even with the lack of regional pathology or virology support, they were able to make the clinicopathologic diagnosis and provide the central laboratory with the required samples. In all of the locations visited, animal health authorities were extremely open and willing to share information on the origin and management of the outbreak. The opportunity to learn from their experience was greatly appreciated. The virus from this outbreak has been

sent to Dr. Erasmus at Onderstepoort, South Africa, who will attempt "fingerprinting" to compare it to other AHS viruses, including viruses from the 1987 outbreak. Veterinarians involved in the culturing of the virus reported that the 1988 virus produces CPE in Vero cells 1 day prior to CPE in MS cells, and that the reverse was true of the 1987 virus. There was a consensus among Spanish field veterinarians that the clinicopathological picture in vaccinated animals was somewhat different from that seen in the earlier nonvaccinated cases. The course of the disease was slightly longer, clinical signs were less clear-cut, and more hemorrhagic lesions were observed.

Recommendations. Due to the laborious and time-consuming nature of complement fixation (CF) testing for AHS, the authors recommend that the development and use of alternate serological tests be more actively explored. They further recommend that APHIS develop and maintain a bank of cell culture-derived AHS vaccines. Initial vaccines produced should be serotypes 4 and 9. Research concerning AHS virus carrier states and reservoirs should be intensified to determine the risk factors associated with the importation of certain equidae and other potential carrier species from AHS countries. U.S. importation procedures currently in effect for horses (60-day quarantine) appear adequate. However, the authors recommend that seronegativity be required for other equidae before release from quarantine.

Additional Cases. Following the trip to Spain, AHS cases were reported 40 km northwest of Las Lomas. An additional 5 ranches and 25 horses were affected near Cadiz in Puerto Real, Chiclana de la Frontera, and Medina Sidonia. These cases occurred in vaccinated horses during late November and early December, or almost a month following the outbreak at Las Lomas. They were attributed to inadequate protection provided by the polyvalent vaccine used in the initial phases of the vaccination campaign. All equines within the existing vaccination zone were revaccinated, using the monovalent Type 4 vaccine.

Another horse died on December 17, in Sanlucar de Barrameda. Sanlucar de Barrameda lies approximately 65 km northwest of the original outbreak area of Las Lomas. Laboratory diagnosis of AHS was completed during January 1989. Veterinary authorities extended the AHS vaccination campaign to include an additional 4,500 to 6,000 equines in the new area. The last death of a horse due to AHS in Andalucia reportedly was on January 30, 1989. During late January in Cadiz Province, one horse died of AHS in Jimena Township, one near Sanlucar de Barrameda, and two near Trebujena. The latter two locations are adjacent to the Las Marismas wetlands. All of these horses had been vaccinated for AHS at least a month prior to their death.

The official toll from the AHS outbreaks in Andalucia during October, November, and December 1988 has been placed at 160 equines, 89 of which died of the disease, with an additional 71 sacrificed upon diagnosis. The last reported case of AHS in Spain occurred December 17, 1988, and, as of January 27, 1989, no other outbreaks have been reported. (Dr. Corrie Brown, Foreign Animal Disease Diagnostic Laboratory, NVSL, ST, APHIS, USDA, Greenport, NY 11944-0848, (516) 323-2500, and Dr. Eric Hoffman, Operations Support, International Services, APHIS, USDA, Hyattsville, MD 20782, (301) 436-8892)

Foreign Animal Disease Update

In South America, during the months of July, August, and September 1988, Brazil reported 371 outbreaks of **foot-and-mouth disease** (FMD) Types O and A; Argentina reported 2 outbreaks of FMD Type O; Venezuela reported 7 herds affected with Types O and A; Ecuador reported outbreaks of both Types O and A; and Colombia reported 119 outbreaks of Types O and A. Colombia, Ecuador, and Panama reported out-

breaks of **vesicular stomatitis** (VS) of Types New Jersey and Indiana. Venezuela also reported cases of VS.

In Europe, Italy reported outbreaks of FMD Type C during July and August. No other cases were reported until November 6, when another outbreak of Type C FMD occurred in the northern province of Reggio Emilia, near the city of Correggio. All 2,555 swine on the premises were destroyed and the area was quarantined. All livestock fairs and exhibitions were canceled. Turkey reported 135 outbreaks of FMD Types O and A during the third quarter of 1988.

In Africa, Cameroon and Libya reported FMD Type O, while Senegal reported outbreaks of FMD, but not the virus type.

In Asia, the Far East, and Oceania, Sri Lanka and Pakistan reported cases of FMD Type O, the Philippines type C, and Nepal Types O and A. Thailand reported cases but not the type. Israel, Kuwait, and Jordan continued to report outbreaks of Type O. Preliminary results indicated that the subtype involved in Israel is related to the O Manisa strain.

The World Reference Laboratory for FMD reported the following diagnostic results for the months of July, August, and September: Type O - Cameroon, Israel, Jordan, Kuwait, Libya, Nepal, and Turkey; Type A - Turkey; Type C -Philippines, and Type Asia 1 - Nepal.

Thirty-one new outbreaks of **rinderpest** (RP) were reported from Sri Lanka during the quarter. Kenya and Uganda also reported RP.

Senegal and Togo in Western Africa reported continuing outbreaks of **peste des petits ruminants**. Togo reported 22,000 animals affected.

Contagious bovine pleuropneumonia (CBP) was reported in the African countries of Namibia and Benin. Kuwait reported 25 cases, while Portugal had 476 new outbreaks and destroyed 1,542 bovines with CBP during the quarter.

Lumpy skin disease was reported in Niger, the Congo, Madagascar, Mali, the Gambia, and Zimbabwe. Madagascar reported 63 new outbreaks.

Outbreaks of **sheep and goat pox** were reported from seven African countries: Algeria, Tunisia, Egypt, Morocco, Mali, Senegal, and Togo. Kuwait, Iraq, and Pakistan also reported cases. In Europe, Greece reported numerous outbreaks, while neighboring Turkey reported 123 new outbreaks of sheep and goat pox affecting 433,311 animals.

South Africa and Zimbabwe both reported cases of **African horse sickness** (AHS). On October 17, 1988, Spain officially announced another outbreak of AHS. The first clinical case was encountered on October 3. By November 3, 20 ranches had reported cases and at least 84 horses had died or were sacrificed. The outbreak occurred in the southern Province of Andalucia and was caused by Type 4 virus, the same type as in 1987 (see accompanying article on AHS in Spain).

In Africa, only Zaire reported cases of **African swine fever** (ASF). In Europe, Italy had 11 outbreaks with 253 swine affected, Portugal had 30 cases affecting 1,181 animals, and Spain reported 117 cases with 5,969 swine that were infected and sacrificed.

During July, August, and September, **hog cholera** was reported in Mexico, Argentina, Ecuador, Paraguay, Chili, Uruguay, Brazil, Taiwan, the Republic of Korea, Sri Lanka,

Malaysia, Yugoslavia, Czechoslovakia, and Belgium.

South Africa reported one case of **Rift Valley fever** during the quarter. Madagascar reported 40 swine died in 8 occurrences of **Teschen disease**.

Twenty-one countries reported outbreaks of untyped **Newcastle disease**: Togo, Botswana, Egypt, Tunisia, South Africa, Madagascar, Congo, Malaysia, Sri Lanka, Iraq, Taiwan, Kuwait, Republic of Korea, Italy, Turkey, Yugoslavia, Mexico, Brazil, Panama, Ecuador, and Paraguay. Even though the virus type or types were not reported, they were presumed to be velogenic.

Velogenic viscerotropic Newcastle disease virus (VVNDV) was identified in samples from Mauritius, South Africa, Pakistan, Malaysia, Paraguay, and the U.S.S.R.

The only country reporting fowl plague during July, August, and September was Senegal in West Africa. (Dr. Eric Hoffman, (301) 436-8892)

Rabbit Disease in Mexico

High mortality in domestic rabbits in Mexico was first reported to the Mexico-United States Commission for the Prevention of Foot-and-Mouth Disease and Other Exotic Diseases (CPA) on January 22, 1989. Under its charge to prevent and control exotic diseases in Mexico, CPA initiated an intensive investigation campaign to determine the incidence of the disease. By February 15, over 200 rabbit breeding establishments had been visited and 160 affected premises had been found. Specimens from affected rabbits were sent to the U.S. Department of Agriculture's Foreign Animal Disease Diagnostic Laboratory at Plum Island, New York, where studies on the causative agent are in progress. A viral agent has been isolated and is being characterized.

A disease that has been associated with similar losses in other countries during the past 4 years has been called viral hemorrhagic disease of rabbits, necrotic hepatitis of rabbits, rabbit viral sudden death, infectious hemorrhagic disease of rabbits, hemorrhagic pneumonia in rabbits, new viral disease in rabbits, hemorrhagic tracheopneumonia of rabbits, x disease of rabbits, and viral hemorrhagic pneumonia of rabbits. The new disease was reported by S. J. Liu and co-workers in China (Anim. Husb. Vet. Med. 16(6):253-255, 1984). Evidently, this disease has spread throughout Europe and some Asian countries, and reportedly has killed millions of rabbits. According to a news report, a total of 32 million rabbits died of the same or similar disease in Italy.

The cause of so-called viral hemorrhagic disease of rabbits has not been clearly identified, but it resembles a parvovirus. The disease is highly contagious and transmission has been reported by aerosols, contact, fomites, meat, rabbit byproducts, insects, and rodents. Attack rates have varied from 30 to 80 percent and mortality rates from 90 to 100 percent of those infected. Incubation periods were from 2 to 3 days. Affected rabbits died suddenly after showing signs of respiratory distress, some with bloody nasal discharge. Postmortem findings were mainly of a severe hemorrhagic disease, primarily of the liver and respiratory system.

Preliminary information suggests that the disease was spread from country to country by shipments of contaminated rabbit meat and infected live rabbits. A shipment of 18,000 kg of frozen rabbit carcasses from China was imported through Laredo, Texas, on November 19, 1988, and was delivered to a supermarket chain outside Mexico City, near the rabbit colony of a veterinary school where some of the first cases of the disease were seen in mid-December 1988. From the suspected primary focus, the disease spread rapidly throughout the greater Mexico City area and into ten neighboring States.

When the extent of the outbreak was realized, CPA recommended that emergency measures be taken to try and prevent further spread and plans be formulated for eventual eradication of the disease. The National System for Emergencies in Animal Health (SINESA) of the Secretariat of Agriculture and Water Resources (SARH) was put into operation by the Secretary and published in the Mexican Federal Register. SINESA is staffed by a multidisciplinary group of SARH officials who were especially trained by the CPA to constitute an emergency task force similar to the U.S. Regional Emergency Animal Disease Eradication Organization (READEO).

The SINESA Task Force initiated eradication operations, beginning with an intensive publicity campaign by the SARH Social Communications Unit, to advise rabbit breeders and the general public about the situation and suggest preventive measures. Extensive surveillance was initiated to determine the extent of the outbreak and help plan for an eventual eradication campaign. Plans include stopping all movements of rabbits from affected areas, quarantines, depopulation of surviving rabbits, cleaning and disinfecting, and repopulating the premises from Government rabbit breeding centers after a waiting period of at least 2 months. The sale of rabbit meat has been prohibited in all affected areas. Depopulation of rabbits was begun at all known foci of the disease. Disease eradication operations are expected to start in the Mexico City Metropolitan area in mid-April, 1989. A total of 100 million pesos was set aside to cover anticipated costs for the first 6 weeks of the campaign. (Dr. John Mason, Co-Director, CPA, USDA, APHIS, American Embassy, Mexico City, Mexico)

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Focus on -- Wesselsbron Virus Disease

Wesselsbron virus (WSL) is widely distributed in Africa, and infection of livestock is probably common in the warmer and moister parts of the continent. Infection is usually mild, except in pregnant and newborn animals, particularly sheep. Outbreaks of disease are recognized when large numbers of susceptible animals are infected within a short period of time. Such outbreaks occur in the drier, temperate livestock farming areas, which are probably marginal for distribution of the virus, and are triggered at irregular intervals of years by exceptionally heavy rains, which favor breeding of the mosquito vectors.

WSL History

The history of Wesselsbron (WSL) virus is interwoven with that of Rift Valley fever (RVF) in southern Africa and has had a marked influence on the manner in which WSL virus is perceived.

The first RVF epizootic to be recognized in South Africa occurred in the summer of 1950-51, and caused severe losses of livestock. Localized RVF epizootics occurred at irregular intervals over the next few years and, in 1955, it was reported that neonatal deaths and abortions were occurring in a flock of sheep in the Wesselsbron district of the Orange Free State province on a farm where RVF vaccine, known to be abortigenic, had been used on pregnant sheep two weeks previously. However, a new virus was isolated from the brain and liver of a decomposed 8-day-old lamb, and was named after the district of its origin. Within a month, the same virus was isolated 800 km to the east from both mosquitoes and the blood of a febrile field worker employed by a medical team investigating arthropod-borne viruses in the northern part of Natal Province. Transmission by mosquitoes was soon demonstrated experimentally. The new agent was identified as a flavivirus.

In 1956, WSL virus was implicated in cases of sheep abortion and neonatal mortality in Kroonstad district. Antibody was demonstrated in lamb sera at a stage when the investi-

gators felt that maternal immunity should have waned. WSL virus was isolated from a lamb in Kroonstad district the following year in the course of entomological studies. WSL virus was isolated from sheep tissue specimens from several farms in the Middelburg district of Cape Province during investigation of an outbreak of disease in 1956 and 1957, as well as from mosquitoes and an entomologist. The clinical picture was considered to be inconsistent with typical WSL virus infection, and it was believed that the outbreak was complicated by other diseases.

Although further outbreaks of WSL virus infection were specifically described in South Africa, the virus was isolated from a moribund cow during entomological investigations in Natal in 1973, organs from a calf in 1974 and another in 1975, and two lambs in 1975. WSL virus was also isolated from the organs of a cow which died during a RVF epizootic in Zimbabwe in 1978. A few other isolations have been made from vertebrates, including man, and numerous isolations have been made from mosquitoes, especially in West Africa.

WSL Vaccine

A partially attenuated WSL vaccine for livestock was developed and marketed in South Africa from 1955 onwards. It was first used on a truly large scale, together with RVF vaccine, during the 1974-75 RVF epizootic in South Africa. During the ensuing months, many cases of teratology were observed.

WSL Antigenic Relationships

The viruses of WSL, yellow fever, Banzi, and Uganda S form a close antigenic group or complex within the flaviviruses. This apparent relationship has not been demonstrated in all studies. Differences in findings may be related to variation in the immunization schedules used to produce antibodies, the susceptibility of the immunized hosts, and antibody test procedures. Strain YM310.66, isolated from a mixed pool of aedine mosquitoes in Cameroon in 1966, shows quantitative differences in cross-serological tests with WSL virus and is regarded as a subtype of the virus.

Disease Characteristics

WSL virus has been isolated from humans on 23 occasions in South Africa, Uganda, Senegal, Nigeria, Central African Republic, and the United States of America. Eleven of the infections were either laboratory acquired, or occurred in field workers collecting mosquitoes. Five other laboratory-associated infections were serologically diagnosed. The virus was isolated from a throat washing in one instance, following a suspected aerosol infection. All other isolations from humans were from blood.

Known incubation periods in human infections ranged from 2 to 7 days. The onset of disease was marked by sudden appearance of fever, rigors, severe headache, tachycardia, anorexia, myalgia, and arthralgia. The headache was variously described as frontal or occipital, but retro-orbital pain was commonly experienced, and some patients suffered photophobia and temporary loss of visual acuity. Several patients experienced cutaneous hyperesthesia of the trunk, arms, or scalp, and two patients were said to have had a "mild" rash over the trunk. Hepatomegaly or hepatosplenomegaly occurred in some patients and was associated with tenderness of the abdomen and occasionally with raised levels of serum alanine and aspartate transaminase. Although patients recovered from the acute illness in 1 to 3 days, and then suffered mild lassitude over a convalescence period of about a week, one experienced muscle pains for a month.

The only isolation of WSL virus from a domestic animal outside of South Africa or Zimbabwe was from the blood of a camel tested during a routine survey in Nigeria. Due to a misunderstanding between reference laboratories, WSL virus reportedly was

isolated from infection in a dog in Botswana, but the agent isolated from the brain of the dog was actually West Nile virus.

In experimental infection, newborn lambs had an incubation period of 1 to 3 days, followed by a short, nonspecific, febrile, viremic illness marked by anorexia and listlessness. Fever was sometimes biphasic. Ten of a total of 27 (37 percent) experimentally infected lambs died within 72 hours of infection (10/27). Older lambs, goat kids, and calves were less susceptible.

Five of a total of 28 (17.8 percent) experimentally infected goat kids died. Adult sheep, goats, and cattle usually developed a mild febrile infection after an incubation period of 2 to 5 days. Up to 20 percent mortality may occur in pregnant ewes with WSL when complications such as pregnancy toxemia, chronic copper poisoning (enzootic icterus), or dystocia occur.

Macroscopic examination of the carcasses of lambs with WSL reveals mild-to- severe icterus and slight to moderate hepatomegaly. The liver is discolored yellow to orange-brown, and sometimes is mottled with congestion. Petechial or ecchymotic hemorrhages may occur on the abomasal mucosa and visceral serosae, and there may be generalized lymphadenopathy.

Histopathological examination of the liver reveals slight-to-extensive, diffuse necrosis of hepatocytes with karyorrhexis, Kupffer cell proliferation, cholestasis, leukocyte infiltration consisting mostly of neutrophils, and proliferation of the bile ducts and histiocytes in the portal triads. Less marked lesions occur in other organs such as the spleen, brain, or lymph nodes. Despite the apparent mildness of infection in adult sheep, histopathological examination of the liver reveals small, multiple foci of necrosis with pronounced, localized Kupffer cell reaction ("retothelial nodules") and mononuclear infiltration of the portal triads. The histopathological lesions are distinct from those of RVF.

Depending on the stage of infection at which investigations are done, virus may be isolated from a variety of tissues, including blood, liver, spleen, heart, adrenal gland, brain, lung, kidney, and lymph nodes of lambs and sheep.

The outcome of infection in pregnant animals is markedly influenced by the stage of pregnancy during which infection occurs. Following experimental infection with nonattenuated WSL virus in late pregnancy, some ewes aborted within a few days. This was considered to be a consequence of fever in the ewes because WSL virus was not isolated from the fetuses. In contrast, abortion that occurred after about 5 days appeared to be due to fetal infection because the virus could be isolated from fetal tissues such as liver and brain. Nevertheless, it should be noted that isolation of WSL virus has never been reported from an aborted fetal specimen from the field.
WSL infection in very early pregnancy may cause embryonic death and unnoticed loss of the conceptus: so-called fetal resorption. Later fetal death may result in abortion or retention and mummification of the fetus.

At a certain stage of organogenesis, fetuses are susceptible to the teratogenic effects of a variety of viruses, including WSL virus. The critical period varies from one animal species to another and is related both to the normal duration of gestation in the species and to the virus concerned. With WSL virus, fetal abnormality was observed when pregnant ewes were infected at 42 to 74 days of gestation. Teratogenesis also occurred when a bovine fetus was inoculated with WSL virus at 115 days of gestation. WSL vaccine virus and wild WSL virus fulfill the criteria for teratogenic agents, in that they do not necessarily cause

severe maternal illness or kill the fetus, but manifest tropism for organs which are not essential to the survival of the fetus, such as the brain.

Depending on the time of onset of teratogenesis, brain lesions range from hydranencephaly, in which the entire cerebral hemispheres may be reduced to fluid-filled meningeal sacs, to porencephalia, involving cystic defects of the cerebrum, microencephaly, and sometimes cerebellar hypoplasia. Arthrogryposis often occurs in association with hydranencephaly, possibly because the lack of trophic effect of nervous stimulation contributes to the hypoplasia of skeletal muscle. Since the control of amniotic fluid volume may depend on the swallowing of fluid by the fetus and its excretion into the allantois via the kidneys, hydranencephaly and lack of development of the muscles of deglutition may contribute directly to the development of hydrops amnii. This, in turn, is associated with prolonged gestation. Ultimately, parturition is marked by dystocia due to an abnormally large fetus and postural defects due to arthrogryposis. Sometimes live progeny are produced. These are often weak and ataxic, refuse to suckle, and die within a few hours or days of birth.

All of the abnormalities that have just been described, and other lesions such as brachygnathia inferior, were observed in sheep specimens obtained from the field in South Africa following large-scale use of the vaccine in 1974 and 1975. Similar abnormalities were also observed in experimentally infected sheep with WSL vaccine or wild WSL virus, or RVF vaccine virus. Hydranecephaly was also encountered in calf specimens, and porencephaly with cerebellar hypoplasia was produced experimentally in one bovine fetus by inoculation with WSL virus.

WSL virus is more pathogenic for pregnant sheep than cattle; fetal death, abortion, fetal abnormality, or neonatal death have been observed in 27 progeny of 43 experimentally infected cattle. In a prospective study, abortion occurred in 1 of 21 pregnant heifers that were infected with WSL virus in the field.

WSL and RVF viruses were not isolated from teratologically affected lambs or calves, regardless of whether they were naturally or experimentally infected. However, WSL virus was isolated from uterine tissue of an experimentally infected ewe. Moreover, inconclusive results were obtained in attempts to demonstrate antibody in the sera of field cases of teratology. WSL antibody was found in 4 of 15 sheep fetuses and lambs, and 2 of 13 bovine fetuses and calves following experimental infection of the pregnant dams. The low prevalence of antibodies was probably due to fetal immunoincompetence at the time of infection.

It can be concluded that, even though the abortifacient and teratological effects of WSL virus can be demonstrated experimentally, it may be difficult to establish an etiological diagnosis by isolating virus or demonstrating fetal antibody in field cases.

Diagnostic Procedures

Intracerebral inoculation of newborn mice probably remains the best method for isolating WSL virus. The lack of susceptibility of weaned mice to intraperitoneal infection forms the basis of a convenient screening test for distinguishing WSL from RVF where the two viruses are encountered together.

Serodiagnosis of WSL virus infection has invariably been based on hemagglutination-inhibition (HAI), complement-fixation (CF), and virus neutralization (NT). In experimental WSL infections of sheep and cattle, homologous antibody became demonstrable by HAI and NT techniques on post-infection day 4 at the earliest, and maximum titers were attained by day 7 to week 3. HAI titers declined to undetectable

levels in 25 months, while minimal NT titers remained detectable at this stage in a constant virus-varying serum dilution technique in cell cultures. CF antibody became demonstrable on day 10 at the earliest and was undetectable by week 12.

Establishing an etiological diagnosis in an outbreak of teratology in livestock can be an intractable problem. The occurrence of numerous congenital defects in a particular breeding season would suggest that an infectious agent is involved and a history of WSL vaccination during pregnancy would raise suspicion. Since fetal deformity becomes evident long after infection has taken place, isolation of WSL virus is unlikely.

WSL Transmission

Isolations of WSL virus were made from pools of *Aedes (Och) caballus sensu lato* (probably *Ae. (Och) juppi)*, and from a mixed pool of *Ae. (Neo) lineatopennis, Ae. (Neo) albothorax (Ae. (Neo) mcintoshi and Ae. (Neo) luridus)*. These mosquitoes were associated with a suspected WSL epizootic in sheep in the Middelburg Cape district of South Africa in 1957, and WSL virus was also isolated from *Ae. (Neo) lineatopennis (Ae. (Neo) mcintoshi)* during the 1969-70 RVF epizootic in Zimbabwe. All other isolations from arthropods appear to have been made in the course of routine surveys, although there is a reference to an epizootic situation in Bozo district in the Central African Republic in 1981, without mention of vertebrate hosts. The isolation of WSL virus from an ixodid tick, *Rhipicephalus muhsamae*, in the Central African Republic in 1984, is potentially of epidemiological importance.

Ae. (Neo) circumluteolus and *Ae. (Och) caballus* s.1. have been shown experimentally to be efficient vectors, suggesting that floodwater breeding aedines of the subgenera *Ochlerotatus* and *Neomelaniconion* play an important role in the circulation of the virus. Transstadial transmission was demonstrated in the argasid tick *Ornithodoros savignyi*, but this has no known epidemiological significance.

It seems feasible that mechanical transmission by biting flies can occur during epizootics, as is suggested to be the case in RVF. In early pathogenicity experiments, laboratory workers apparently were infected either from handling infected tissues and cultures or from aerosols.

WSL virus did not appear to be highly contagious among sheep. In several instances, newborn lambs apparently failed to acquire infection from ewes undergoing the acute phase of illness. More recently, 3 of 8 newborn lambs were infected when left in contact with infected contemporaries. In two instances, transmission may have occurred when intravenous needles were contaminated by gloves worn to bleed both infected and noninfected lambs. A third lamb appeared to acquire infection from close contact with its infected twin, which suckled the same ewe.

WSL Geographic Distribution

WSL virus has been isolated from vertebrates or arthropods in South Africa, Zimbabwe, Uganda, Kenya, Nigeria, Central African Republic, Senegal, Cameroon, Ivory Coast, and Thailand. In addition, the results of antibody surveys suggest that the virus was also in Mozambique, Botswana, Namibia, Angola, and possibly Madagascar. Information is lacking for many countries in Africa, but from the distribution of the aedine mosquitoes associated with WSL virus, it can be surmised that the virus occurs more widely than is at present realized.

WSL Incidence

There is a high prevalence of antibody to WSL virus in the warmer and moister parts of southern Africa, namely, northern Natal Province of South Africa, and many parts of Mozambique and Zimbabwe, which are largely cattle farming areas.

The high prevalence of antibody to WSL virus in northern Natal, Mozambique, and Zimbabwe suggests that there is a high incidence of infection. Indeed, from serial monitoring of cattle in Zimbabwe, it may be deduced that at least one-eighth of the cattle become infected each year. Moreover, in the absence of harsh winters, infection appears to occur throughout the year. WSL infection is common, but the disease is rarely recognized in what can be described as epidemiologically stable areas. The situation in these enzootic or "hyperenzootic" areas appears to be that many animals are immune by the time they are bred. As a result, they do not suffer abortifacient or teratogenic primary infection during pregnancy and are able to confer maternal immunity on their offspring. Furthermore, since infections occur throughout the year, there is unlikely to be a sudden upsurge of primary infections at a critical stage of the livestock breeding cycle, which would result in sharp outbreaks of abortion, fetal abnormality, or neonatal death. The virus is, in any event, less pathogenic for the cattle and goats which vastly outnumber sheep in these areas. Human infections, usually mild, are probably often mistaken for "malaria."

On the inland plateau of South Africa, where the main mosquito vector is *Ae. (Och) caballus-juppi*, drier conditions and winter frosts produce irregular, short-lived outbreaks of WSL infection that depend on episodes of heavy rain. The lower prevalence of immunity and the innate susceptibility of sheep must further contribute to the occurrence of periodic outbreaks of abortion, fetal abnormalities, and neonatal deaths in this area. Here, infection is more infrequent, but disease is more readily apparent.

The link, which was originally suggested to exist between outbreaks of RVF and WSL infection, has been borne out to the extent that isolations of WSL virus from livestock in southern Africa have occurred most often at times of heavy rainfall when RVF virus was also more active. However, it was established serologically in Zimbabwe that WSL infection is far more frequent and geographically widespread than RVF infection.

The abundance of rainfall in South Africa appears to follow an 18-to-20 year cycle, and it has been pointed out that past outbreaks of RVF and, hence, WSL infection, have coincided with periods of heavy rainfall and can be expected to occur again with the heavy rains projected to occur at the end of the present decade.

The pattern of distribution in Kenya appears to be similar to that in South Africa, with WSL antibody being common on the coast, but with West Nile (WN) antibody predominating in the interior. Prevalence of antibody to WSL virus exceeded prevalence of antibody to other flaviviruses in Uganda, the Caprivi Strip of Namibia, and parts of Angola. Elsewhere in Africa, antibodies to WN, YF, dengue, and Zika viruses were generally more common than WSL antibody, despite the fact that there have been multiple isolations of WSL virus in West Africa. A livestock disease problem has been described for WSL only in South Africa.

WSL Predisposing Factors

Sheep are more susceptible to WSL virus than goats or cattle. The virus is more pathogenic for pregnant and newborn animals. Pregnant sheep are at increased risk during RVF epizootics when they are inoculated with live, partially attenuated RVF and WSL vaccines. These vaccine viruses retain abortifacient and teratogenic properties. Infection is more severe when there is underlying liver disease from pregnancy toxemia in sheep, chronic copper poisoning, and heptotoxic plant poisoning. These problems occur in parts of the sheep farming areas of the Cape Province of South Africa. Human infections are recognized most frequently in laboratory workers.

WSL Overwintering

By analogy with the mechanism proposed for RVF, it is possible that WSL virus may be overwintered and perpetuated through transovarial transmission in aedine mosquito vectors. Such a mechanism seems particularly likely on the inland plateau of South Africa where harsh winters suppress adult mosquito activity. Eggs of aedines can withstand the long periods of desiccation between periods of heavy rainfall on the plateau. Virus activity appears to occur year-round in the warmer and moister parts of southern Africa, so, theoretically no special mechanism is required for overwintering and perpetuation of the virus in such areas. Infected migrating birds may play a role in disseminating WSL virus from areas where it is enzootically active, but the available evidence suggests that birds are refractory to the virus.

WSL Prevention and Control

WSL may be prevented by vaccination. The present freeze-dried veterinary vaccine is prepared from a strain of WSL virus which has undergone 110 passages in primary lamb kidney cell cultures and 3 in a line of baby hamster kidney (BHK21) cells. The vaccine is sold for use on nonpregnant sheep and cattle in winter or early spring before mosquitoes become active. Immunity is considered to be durable, and revaccination is not recommended. There is no human vaccine.

General recommendations made to farmers concerning the control of WSL and other arthropod-borne infections of livestock include drainage or treatment of mosquito breeding sites, housing sheep at night, and moving stock away from low-lying, poorly drained areas to high, wind-swept grazing during outbreaks of the disease. (Professor R. Swanepoel, National Institute for Virology, University of Witwatersrand, Private Bag X4, Sandringham, 2131 South Africa)

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